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Update on Development and Growth Regulation

Sensitivity Thresholds and Variable Time Scales in Plant Hormone Action[1]

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Plant growth regulators are involved in major developmental transitions, such as flowering, embryogenesis, or dormancy, and in real-time responses to environmental conditions, such as adjustments in growth rates or stomatal conductance. In addition, it is generally the case that only specific cells or tissues in the plant are responsive to a particular regulatory factor. For example, only certain cells at or near the abscission zone express cellulase when exposed to ethylene (del Campillo et al., 1990); aleurone cells of cereal grains respond specifically to GA by synthesizing α-amylase (Jacobsen and Knox, 1973); stomatal guard cells are dramatically responsive to ABA (MacRobbie, 1991); and transcriptional responses to auxin vary markedly among different cell and tissue types (Gee et al., 1991). The wide range of hormonal responses evident among individual cell types and the different developmental and physiological time scales on which hormones act raise two questions that will be addressed here. (a) How is the variation in hormonal sensitivity or content among cells or tissues integrated into developmental patterns? (b) What is the relationship of hormonal sensitivity or content to the timing of developmental or physiological events? Recently, new insights into these fundamental questions have begun to form into a general model of plant hormone action. Our objective here is to outline a novel conceptual framework that integrates variation in both hormonal sensitivity and content with an expanded view of biological time.

POPULATION VARIATION IN SENSITIVITY OF PLANT CELLS AND TISSUES

One of the most fundamental experiments in plant hormonal physiology is the dose-response curve. Plant hormones characteristically exhibit wide activity ranges, often spanning 4 orders of magnitude in concentration (Trewavas, 1991). For example, GA will induce germination of many dormant seeds, and dose-response curves can be constructed for final germination percentage as a function of GA concentration. At a low GA concentration few seeds germinate, but with increasing concentrations more and more seeds are capable of initiating radicle growth (Fig. 1A; Ni and Bradford, 1993). Thus, while additional seeds are induced to germinate as the GA concentration increases, an individual seed either does or does not complete germination at a given GA dose. Since sensitivity can be defined as the response induced by a concentration, the dose-response curve represents the distribution of different individual seed sensitivities to GA within the population. For the case of a GA-deficient tomato (*Lycopersicon esculentum* Mill.) mutant, a more than 400-fold greater GA concentration was required to induce germination of the least sensitive 10% of the seed population compared with the most sensitive 10% (Fig. 1B; Ni and Bradford, 1993). Although the dose-response curve for the population spread over several orders of magnitude in GA concentration, the concentration difference between dormancy and germination was small for any individual seed.

These concepts are equally applicable to individual cells in tissues. Hooley (1982) showed that aleurone protoplasts from wild oat (*Avena fatua* L.) grains exhibited a logarithmic increase in α-amylase secretion as GA_3 concentration increased from 10^{-12} to 10^{-7} M (Fig. 2A). The implicit assumption is often made that this represents a quantitative response in enzyme secretion per protoplast as the GA concentration increases. This may occur, but it is equally plausible that individual protoplasts vary in sensitivity to GA, and that increasing GA concentrations recruit additional members of the protoplast population into the secreting mode. Jacobsen and Knox (1973), using immunolocalization of α-amylase secretion in barley (*Hordeum vulgare* L.) aleurone layers, noted that "not all cells of the aleurone layer responded to GA_3 simultaneously. The middle layer of cells appeared to produce amylase ahead of the other cells. . .so that any assay of amylase synthesized by an aleurone layer represents the sum of the enzyme productions of individual cells at different stages of induction rather than a simultaneous response."

More recently, it has been demonstrated that the percentage of individual barley aleurone protoplasts actively secreting amylase in response to a saturating dose of GA_3 increased slowly to a maximum of 50 to 60% over 10 h (Hillmer et al., 1993). Furthermore, if a lower GA concentration was used, the percentage of secreting protoplasts also decreased

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Abbreviations: GA_b, base or threshold GA concentration; $IAA_b(c)$, base or threshold IAA concentration for a given cell.

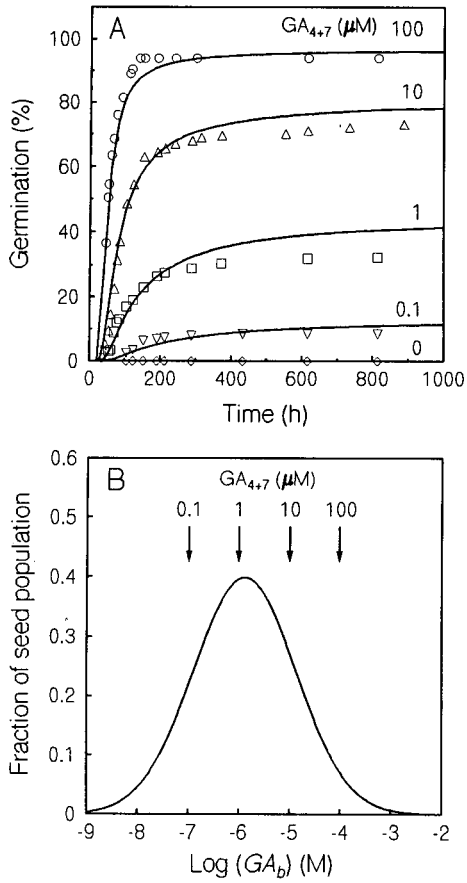

Figure 1. A, Germination time courses of GA-deficient *gib-1* mutant tomato seeds that had imbibed a range of GA_{4+7} concentrations. In the absence of applied GA_{4+7}, the seeds are completely dormant. Increasing GA_{4+7} concentrations both shorten the time to germination and increase the final germination percentage. The solid curves are the time courses predicted by a "GA time" model (e.g. Eq. 1) based on the sensitivity distribution shown in B. B, The distribution of threshold sensitivities to GA_{4+7} (GA_b, or the minimum GA_{4+7} concentration required to stimulate radicle emergence) among individual seeds in the population. The normal curve indicates the relative frequency in the seed population of a given value of GA_b. The time to germination is inversely proportional to the amount by which the current level of GA_{4+7} exceeds the GA_b value for a given seed. The arrows at the top indicate the GA_{4+7} concentrations applied in A; only seeds with thresholds lower than the applied concentration will germinate, resulting in the time courses shown (adapted from Ni and Bradford, 1993).

(S. Gilroy, personal communication). Thus, both an increase in secretion per responding protoplast and the recruitment of additional protoplasts would contribute to the dose-response relationship observed with populations of protoplasts (Hooley, 1982). An individual cell might respond in a concentration-dependent manner only over a relatively limited range of GA concentrations (Fig. 2C), whereas the broad dose-response curve would reflect the variation in GA sensitivity within the cell population (Fig. 2B). A skewed rather than a normal distribution of threshold sensitivities among protoplasts could contribute to the logarithmic increase in α-amylase secretion. Bud growth, shoot and root regeneration, root and stem hair formation, vascular tissue differentiation, abscission, ripening, and flowering are examples of threshold (all-or-none) phenomena that can be regulated by an inducing stimulus in a dose-dependent fashion (Trewavas, 1991). Again, these dose-response characteristics are likely to result from sensitivity variation among the individual cells that constitute the responding tissue.

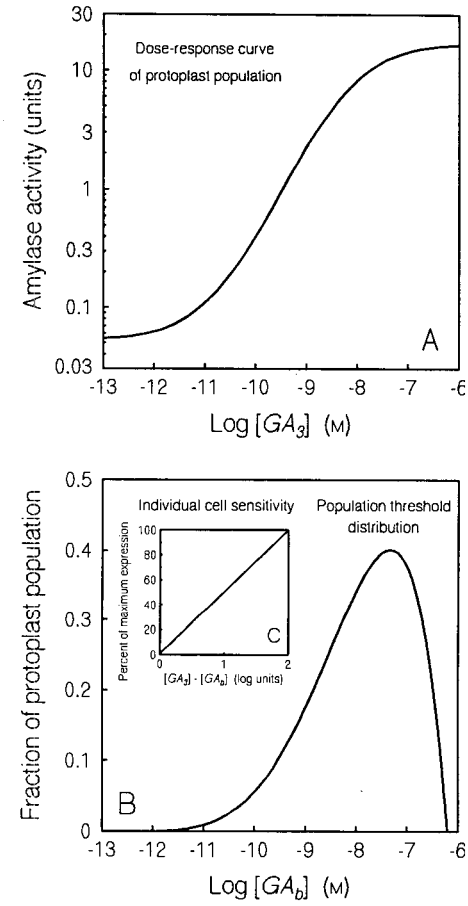

Figure 2. A, Dose-response curve of α-amylase synthesis in wild oat aleurone protoplasts in response to GA_3 (adapted from Hooley, 1982). Note logarithmic scales for both GA_3 concentration and α-amylase activity. B, Theoretical distribution of GA sensitivity thresholds among individual protoplasts that could account for the dose-response characteristics shown in A. The skewed distribution (represented by the β distribution; see Lloyd et al., 1992) would result in many more protoplasts being stimulated to synthesize α-amylase when the GA_3 concentration exceeded about 10^{-9} M, resulting in the logarithmic increase in enzyme activity. C illustrates that the dose-response curve of an individual protoplast might be log-linear with GA_3 concentration over a relatively small concentration range. The x axis indicates the log molar GA_3 concentration in excess of the log GA_b threshold for a given protoplast. The combination of individual protoplast responses over a relatively restricted concentration range with population variation in the threshold concentration could result in the wide dose-response curve shown in A.

PLANT HORMONES MODIFY BIOLOGICAL TIME (BIOTIME)

In addition to an appreciation of sensitivity variation, a flexible view of time may be integral to understanding plant development. Unlike some animal systems in which developmental time is tightly scheduled (Cooke and Smith, 1990), plants often measure environmental time for days, weeks, months, or years before initiating a change in program. Plant hormones can influence the rates at which physiological processes occur. Soon after the discovery of auxin, Cholodny (1931, cited in Went and Thimann, 1937) concluded that the hormone "appreciably accelerates the rate of development and (correspondingly) shortens the duration of the life cycle of each cell." Recently, this concept has been analyzed in a manner analogous to the effects of temperature on biological developmental rates (Bradford et al., 1993). The rates of many plant processes across temperatures can be normalized to a single common rate on a thermal time scale. Thermal time is accumulated as the product of the degrees in excess of a threshold, or base, temperature (T_b) multiplied by the duration at the ambient temperature (Fig. 3A). As the difference between the ambient temperature and the T_b increases, the clock time required to achieve a given developmental step decreases in inverse proportion. One can view developmental or biotime scales as expanding or contracting relative to clock time as the temperature decreases or increases. Similar normalized time scales may also be appropriate for water availability, growth regulators, light, nutrients, and other primary environmental and physiological factors that influence plant development (Ni and Bradford, 1993). Biological time for plants may shrink or stretch in proportion to the relative strength of inputs regulating the rate of approach toward a developmental or growth event. In addition, biotime may be passing at different rates among individual cells or tissues of a single plant (Freeling, 1992).

An example of the application of biotime to hormonal regulation is the accurate modeling of GA-dependent germination rates using a concept of "GA time" analogous to thermal time (Figs. 1 and 3; Ni and Bradford, 1993). As described above, individual seeds possess base GA (GA_b) values, or sensitivity thresholds, which set the minimum GA concentration required to induce radicle emergence. In addition, as the amount by which the GA concentration exceeds GA_b increases, the time to germination decreases proportionately (Fig. 3B). The GA_b values vary among individual seeds (Fig. 1B), but the difference between the GA_b value for a given seed and the actual GA concentration, multiplied by the time to radicle emergence, is a constant for all seeds in the population. Seed germination kinetics in response to ABA and water potential can likewise be predicted using concepts of ABA time and hydrotime (Bradford, 1990; Ni and Bradford, 1992, 1993). For an individual seed, developmental time progresses rapidly or slowly, depending on the extent by which a given regulatory factor differs from its base level. Developmental time courses at a range of regulator concentrations can be normalized on a common biotime scale (Ni and Bradford, 1993), just as developmental processes at a range of temperatures can be normalized on a common thermal time scale.

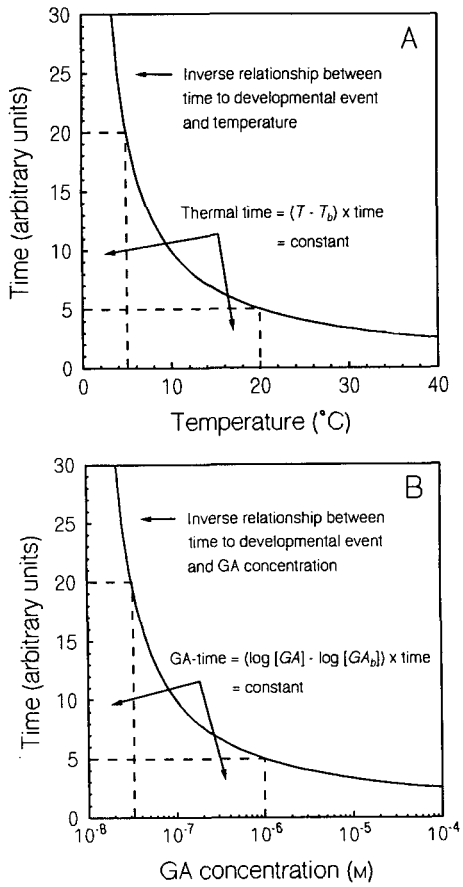

Figure 3. Comparison of the concepts of thermal time and "GA time". A, The rates of many biological processes are inversely proportional to the degrees (T) in excess of T_b for that process. The curve illustrates this relationship for the case where T_b = 0°C and the total thermal time required is 100 degree-days, degree-hours, or other appropriate thermal time units. The product of $T - T_b$ multiplied by the time to the biological response is a constant (i.e. the areas of the dashed rectangles are equal). This allows developmental processes at different temperatures to be normalized on a common thermal time scale. B, Similarly, the concept of hormone time or biotime postulates that the rates of developmental processes accelerate or decelerate in proportion to the amount by which the level of a developmental regulator (GA in this example) exceeds its GA_b. Again, the product of the regulator concentration (log[GA]) in excess of a sensitivity threshold (log[GA_b], where GA_b = 10^{-8} M in this example) multiplied by the time to the developmental event is a constant. As for thermal time, this allows GA-regulated developmental events at different GA concentrations to be normalized on a common GA time scale (Ni and Bradford, 1993).

A POPULATION-BASED THRESHOLD MODEL FOR HORMONE ACTION

These concepts can be described by a simple mathematical model that illustrates the relationships among sensitivity thresholds, hormone levels, and developmental time scales. The model assumes that each cell in a responding population has a threshold or base response level (X_b) for a given factor X that determines its sensitivity to that factor. The threshold values can vary among individual cells, designated by $X_b(c)$.

In many cases, $X_b(c)$ will be a normal distribution (e.g. Fig. 1B), but it need not be (cf. Fig. 2B). The time to a given developmental response for each cell (t_c) is inversely proportional to the difference between the current factor level and the threshold for that cell [$X - X_b(c)$]. This means that the product of this difference multiplied by t_c is a constant, designated θ_X. The model can then be expressed as:

$$\theta_X = [X - X_b(c)]t_c \quad (1)$$

where θ_X is the factor time constant, X is the current factor level, $X_b(c)$ is the distribution of thresholds among cells, and t_c is the response time. It is apparent that if θ_X is a constant, then t_c will be reduced proportionately as the concentration of X increases above $X_b(c)$. In Figure 3, the value of the time constant is represented by the equal areas of the dashed rectangles, and the solid curves show the inverse relationship between time and the temperature or concentration above their respective thresholds. Once the values of the time constant and the threshold distribution are known, the response kinetics can be predicted at any level of the factor, as illustrated by the predicted responses of germination to GA concentration (solid curves in Fig. 1A). A convenient feature of the model is that it also can account for inhibitory effects of hormones simply by defining $X_b(c)$ as the concentration that just blocks the response and changing the sign of the time constant (Ni and Bradford, 1992, 1993). This model resolves the conflict between concentration and sensitivity as critical factors in hormonal regulation (Trewavas and Cleland, 1983), since it is driven by the difference between the concentration and the threshold sensitivity rather than the absolute value of either. Variation in either parameter has equivalent effects on developmental timing. We believe that this model can describe many aspects of development that involve threshold phenomena and populations of responding cells.

PREDICTABLE PATTERNS CAN BE GENERATED BY STOCHASTIC PROCESSES IN CELL POPULATIONS

The work on seed germination indicates that the thresholds of individual seeds vary in a normal distribution, resulting in an ordered and consistent germination pattern among the population. Similarly, the loss of viability in a seed population occurs according to a negative cumulative distribution as individual seeds die (Ellis and Roberts, 1981). However, Roberts (1972) has shown how this regular pattern of viability loss could be explained on the basis of the completely random loss of function of as few as 20 out of 100 key cells in each seed. Another example of stochastic processes in cell populations can be found in studies on cell replication in suspension cultures. Mitotic cells can remain in a nonreplicating mode (G_1 phase) for an indeterminate time (Smith and Martin, 1973). Cells cross a threshold and leave G_1 to carry out the all-or-none process of cell division. The frequency (or transition probability) with which cells leave G_1 is determined by the environmental conditions and the particular cell type. All mitotically competent cells have an equal probability of leaving G_1 at any time, and this probability may not increase with time spent in G_1 (Smith and Martin, 1973). Individual cell entry into replication is stochastic and is best understood as analogous to radioactive decay. This stochastic behavior on a cellular level can nonetheless result in characteristic and predictable cell division patterns on a population level. For example, it is impossible to know which radioactive nucleus will disintegrate next, but the overall decay pattern of an isotope is highly predictable. Recent results in mammalian neuronal development also support the concept that stochastic variation among individual neurons, combined with relatively minor gradients in environmental inputs, can have large yet predictable consequences for developmental patterns (Stryker, 1994).

Are stochastic processes involved in hormonal regulation? When root segments are treated with auxin, individual pericycle cells enter mitosis, eventually forming lateral roots. The numbers of roots formed depends on the auxin concentration. Therefore, there is a stochastic distribution of auxin sensitivities in the pericycle cell population, and additional cells are induced to enter mitosis as the auxin concentration exceeds their individual thresholds. In addition, the numbers of lateral roots formed also depends on the duration of auxin treatment (Blakeley et al., 1972). Short exposures trigger relatively few cells, but longer exposures trigger more cells to enter mitosis. This time-dependent sensitivity is a useful way of adjusting the numbers of lateral roots to either the duration or the strength of the signal, and is consistent with the model of Equation 1. Another familiar example of this type of behavior is the reciprocity relationship in photobiology, where equivalent photon doses (intensity × time) have equivalent developmental effects. The model advocated here of variation in sensitivity thresholds combined with a reciprocal relationship between dosage and response time (Eq. 1) is readily applicable in photobiology (Frankland, 1975).

A stochastic model of sensitivity thresholds might also be appropriate to describe the responses of individual stomata to ABA or environmental factors, in which nonuniform closure or "patchiness" has been observed (Terashima et al., 1988). Experimental evidence indicates that stomata do behave as populations with widely varying individual response thresholds at both the leaf and cellular levels (Laisk et al., 1980; McAinsh et al., 1992). Recent models of stomatal behavior explicitly incorporate population variation in stomatal aperture into their calculations (Cheeseman, 1991; Lloyd et al., 1992). Individual guard cell pairs might vary stochastically in their response thresholds for a number of regulatory factors (ABA, CO_2, light, humidity, turgor, etc.), providing the leaf with a wide array of possible gas-exchange states brought about by the interaction of the local internal and external environments with the physiologically determined sensitivity threshold distributions.

SYNCHRONIZATION CAN RESULT FROM ALTERED THRESHOLDS, CONCENTRATIONS, OR BIOTIME SCALES

Growth regulators can also synchronize all-or-none developmental processes. GA (and other) treatments, for example, can synchronize seed germination (Trewavas, 1988; Ni and Bradford, 1993), whereas ethylene can synchronize the ripening of fruits (McGlasson and Pratt, 1964) and dehiscence of abscission zones (Lipe and Morgan, 1972). There are at

least three ways that synchronization could be achieved in the present model.

Treatments that synchronize the developmental event may simply lower the response threshold. This would uniformly increase the likelihood that a given cell will experience an environment or signal exceeding its sensitivity threshold, and will therefore undergo the developmental transition. In a population that exhibits a distribution of response thresholds, narrowing the variation among cells would also synchronize the response across all regulator levels, as the spread in response times is determined by the spread of the threshold distribution. Alternatively, synchronization on a clock time scale can be achieved by expanding or shrinking the biotime scale without affecting the underlying threshold variation. Even if the relative variation among individuals remains the same, an event that happens over a shorter duration (e.g. due to a higher hormone concentration) will appear to be more synchronous than one that extends over a longer period. As an analogy, the finishing times of athletes in a 100-meter race are more synchronous in clock time than the finishing times for a 1000-meter race, even if the relative abilities of the participants are identical in both races. Thus, shrinking a biotime scale relative to clock time by speeding up a process will result in synchronization even without any change in the underlying variation in response thresholds.

These alternatives can be illustrated by the case of ethylene promotion of the respiratory climacteric in fruits (Trewavas, 1991). Ripening of detached fruits is marked by a climacteric rise in CO_2 evolution, the onset and duration of which can be shortened by treatment with ethylene (McGlasson and Pratt, 1964; Saltveit, 1993). Individual fruits must reach a mature stage before treatment with ethylene is effective, indicating that ethylene sensitivity increases (the response threshold decreases) during fruit development. Even within a single fruit, the climacteric is the composite activity of millions of fruit cells that may vary in their developmental stage or sensitivity to ethylene. Supplemental ethylene will exceed the thresholds of many additional cells, advancing and synchronizing the respiratory response. Ethylene might also alter the tissue threshold distribution, making all cells more sensitive to the gas and thereby achieving the same effect. The induction of enzymes responsible for ethylene synthesis would work similarly by elevating endogenous ethylene concentrations. Ethylene could also have a purely kinetic effect, compressing the developmental time scale over which the normal climacteric pattern will occur. This "ethylene time" concept is supported by the inverse relationship between ethylene concentration and the minimum exposure time required to initiate ripening (Inaba and Nakamura, 1988), consistent with the model of Equation 1.

APPLICATION OF SENSITIVITY AND BIOTIME VARIATION TO GROWTH ANALYSIS

Can we apply these important concepts to growth, even though growth is not usually considered an all-or-nothing phenomenon? Plant growth is dependent on the generation of new cells in the meristem and their subsequent expansion in the elongation zone. In the meristem, the transition from cell division to elongation is an all-or-nothing step for individual cells. Mitotic cell numbers diminish progressively with distance from the meristem and submeristematic regions contain mixtures of dividing and elongating cells. Since mitotic cells expand at a much slower rate than elongating cells, tissue growth rates in this transitional region progressively increase as mitotic numbers and thus growth constraints diminish. The opposite effect presumably happens as the percentage of maturing cells increases in the distal regions of the growth zone and the growth rate declines. The total length of the growth zone and the maximum rate of expansion are sensitive to hormonal and environmental factors (Saab et al., 1992; Ishikawa and Evans, 1993). Subpopulations of cells may differ in their growth characteristics and hormonal sensitivity depending on their position within the meristematic and growth zones of specific tissues. In the maize (*Zea mays* L.) seedling, for example, ABA has contrasting effects on the root and shoot growth zones, and the sensitivity to ABA action varies even within each growth zone (Saab et al., 1992). Individual plants also vary widely in growth rates and final size even under identical conditions. Coleoptile terminal heights can vary up to 5-fold in barley seedlings; the different heights are associated with different growth patterns, and the smallest coleoptiles are insensitive to auxin (Liptay and Davidson, 1971). Varieties of cultivated pea are available with genetically defined terminal heights anywhere from 0.3 to 2 m (Sutcliffe, 1977). The growth of many of these is GA dependent, and the primary difference among them is in internode length. Even within a specific cultivar, different internodes vary in length in a reproducible fashion. Thus, variation in hormone-regulated growth patterns is present at the cell, tissue, organ, individual plant, and species levels, and our hypotheses of hormone action must accommodate population phenomena at each level of organization.

Data on the inhibition and adaptation of maize root growth to auxin (Ishikawa and Evans, 1993) can be used to illustrate how a population model such as that in Equation 1 can be applied. The variation in local growth rates along the expansion zone exhibits an approximately normal distribution. The growth zone can be considered to represent a population of cells that vary in their current expansion rates and in their sensitivity to regulatory factors. Each individual cell may have its own base thresholds for auxin, GA, and other factors that change as the cell moves through the growth zone (Gotô and Esashi, 1974). Curve a in Figure 4A approximates the growth rate distribution through the elongation zone of control maize roots (Ishikawa and Evans, 1993). The dashed curve in Figure 4B shows a corresponding theoretical threshold (sensitivity) distribution of cells at different positions in the growth zone. The y axis in this case is the IAA concentration required to inhibit expansion of a given cell [$IAA_b(c)$]. The threshold distribution predicts that cells near the midpoint of the expansion zone will require higher IAA concentrations for inhibition than cells at the extremes. The height of the curve at any point (e.g. Δa in Figure 4B) indicates the difference between the IAA threshold and the endogenous level, and is proportional to the cell growth rate at that same position. This can be seen by rearranging Equation 1 in the form of a rate ($1/t_c$),

$$1/t_c = [X - X_b(c)]/(-\theta_X) \qquad (2)$$

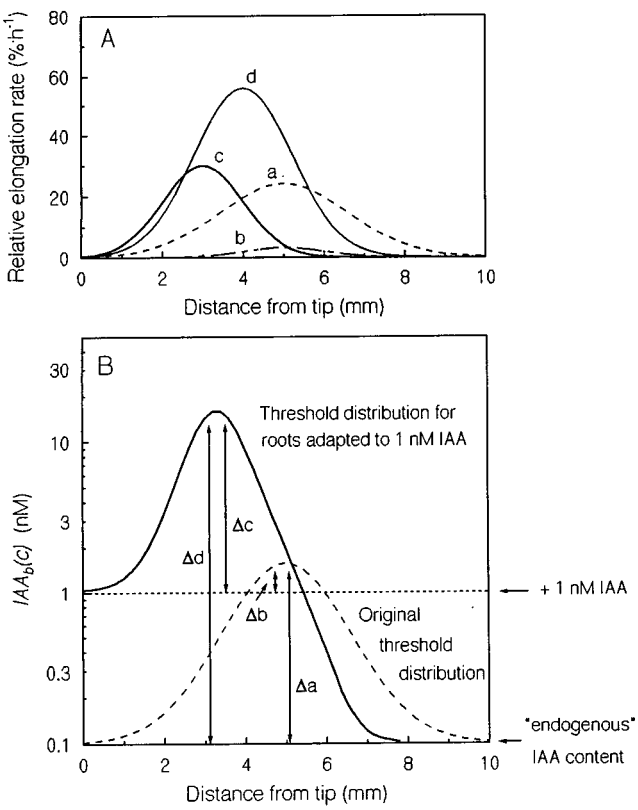

Figure 4. A, Relative elongation rates at different distances from the tip of growing maize roots. The curves are normal distributions that approximate the actual distributions reported by Ishikawa and Evans (1993). The conditions are a, control roots; b, roots soon after the addition of 1 nM IAA to the growth medium; c, roots that have recovered growth in the continuous presence of 1 nM IAA; d, enhanced growth of adapted roots from c after removal of exogenous IAA. B, Theoretical IAA sensitivity distributions that can account for the growth patterns in A. The *y* axis represents the threshold or base IAA concentration required to inhibit growth of a given cell [$IAA_b(c)$]. The dashed line represents the sensitivity distribution of control roots, and the solid line shows the sensitivity of roots adapted to the presence of 1 nM IAA. The horizontal dotted line indicates the addition of 1 nM exogenous IAA; cells with IAA_b values below this line are prevented from expanding. The double-headed arrows (Δa, Δb, Δc, Δd) indicate the difference between the current physiological IAA level and the inhibitory threshold level under conditions corresponding to curves a, b, c, and d in A. These differences are directly proportional to growth rate at a given location in the growing zone.

which shows that as the IAA concentration (X) approaches the inhibition threshold of a particular cell [$X_b(c) = IAA_b(c)$], the growth rate of that cell will decline proportionately until growth stops when $IAA = IAA_b(c)$. (The negative sign on the time constant indicates that the factor inhibits or slows the response time.) Note that the result would be identical if the thresholds were constant for all cells, but the IAA concentration varied with distance from the tip, since the rate is sensitive only to their difference.

Responses to applied IAA, however, suggest that threshold differences are the more likely explanation. When 1 nM IAA was supplied to the growing roots (dotted horizontal line in Fig. 4B), only cells whose inhibition thresholds exceeded 1 nM continued to grow (curve b in Fig. 4A; Ishikawa and Evans, 1993), but their growth rates were much reduced (indicated by the small Δb in Fig. 4B). After about 90 min, however, growth resumed at the original rate even in 1 nM IAA but with a growth distribution shifted nearer the root apex (curve c in Fig. 4A). This can be explained if the adaptation process acts by increasing the inhibition thresholds of cells in the apical region of the growth zone to higher IAA concentrations, thus reestablishing the differential between the threshold [$IAA_b(c)$] and the actual concentration (solid curve in Fig. 4B). Under these conditions, Δc approximates Δa, and growth resumed at near its initial maximum rate. If the exogenous IAA was removed at this time, there was a large overshoot in growth rate before it returned to the original rate (curve d in Fig. 4A; Ishikawa and Evans, 1993). This is predicted by the threshold model, since the adapted cells will now have inhibition thresholds much in excess of the endogenous IAA concentration (Δd in Fig. 4B), which will translate into a proportionately rapid growth rate (Eq. 2). Additional data on the growth distributions in gravistimulated roots in the presence and absence of auxin (Ishikawa and Evans, 1993) can also be interpreted in a manner consistent with this model, assuming that the orientation to the gravity vector can alter either the IAA concentrations or the threshold distributions of the upper and lower tissue halves (Evans, 1991). A recent model of gravitropic sign reversal is also based on cell-to-cell variation in perception or response and the possibility of developmentally dependent changes in threshold distributions (Myers et al., 1994).

A further prediction of this analysis is that the auxin sensitivity distribution through the growth zone may be determined at or near the meristem, thereby specifying the growth trajectory of a given cell and its response to regulatory signals. Woodstock and Skoog (1962) long ago reported that the majority of RNA synthesis associated with growth zones occurred primarily at or near the meristem. Modern hybridization techniques have confirmed that expression patterns of at least some genes are consistent with transcriptional activity being concentrated in the apical meristematic region and declining through the growth zone (Mason and Mullet, 1990). Given finite lifetimes of individual mRNA and protein molecules, the transcription of growth-related or threshold-determining mRNAs only near the meristem, their translation into protein, and the subsequent decline in mRNA and protein levels could readily explain the consistent shapes of the growth rate distribution patterns. Under constant conditions, each cell in the growth zone may have a similar biotime trajectory, but when conditions change rapidly, the growing zone may be composed of successive cohorts of cells with different initial mRNA or protein complements and therefore different biotime trajectories. The numbers of cells in the growth zone, the length of time that a given cell is in its growth phase, the maximum expansion rate, and the sensitivity to regulatory factors could all be determined by the initial complement of mRNAs transcribed in a cell, their rate of translation into protein, and the turnover rates of both the mRNAs and the proteins. These effects at the individual cell level would have multiplier effects on tissue growth rates,

resulting in wide dose-response curves and high sensitivity to regulatory factors.

BIOLOGICAL VARIATION AND STOCHASTIC PROCESSES MAY BE HIDDEN BY AVERAGING METHODOLOGIES

These few examples suggest that sensitivity variation and stochastic processes may be widespread in plant developmental regulation. They may, in fact, be a fundamental way that plants cope with a stationary existence in a stochastic environment. Variation in response thresholds of individual cells in tissues may provide a built-in plasticity to match the short-term chaotic variation in environmental conditions, just as genetic variation buffers the species against long-term climate change. The presence of calcium-dependent and -independent mechanisms of stomatal closure or growth substance-dependent and substance-independent pathways in development are perhaps indications of this plasticity (Trewavas, 1991). The significance of stochastic processes usually goes unremarked and most developmental processes are conceived as purely graded phenomena. We believe that in numerous cases this view is incorrect; stochastic variation among cells is simply disguised by the averaging of cellular responses in tissues. At the biochemical level, it is believed that ligands and receptors interact by chemical equilibrium, but the interaction of a single receptor molecule with a ligand is a quantal phenomenon and stochastic in basis. Calcium signaling commences with the opening of calcium channels, but opening of individual channels is stochastic and governed by probability. Since very few channels need open to signal individual cells, processes controlled both by calcium and a few progenitor cells will surely exhibit pronounced stochastic characteristics. When the physiological response is averaged over thousands of receptors, over millions of cells and usually numerous plants, its stochastic basis is deceptively obliterated. When fewer receptors, cells, or plants are examined, a more variable stochastic realm is uncovered (McAinsh et al., 1992; Hillmer et al., 1993), which is often taken to be experimental error (Spence, 1987).

Experimental designs can confound attempts to quantify and understand stochastic processes in plant responses by minimizing or pooling variation within the system examined. For example, the activity of endo-β-mannanase in tomato endosperm tissues varied over 1000-fold among individual seeds within a single seed lot (Dahal et al., 1994). The existence of this magnitude of variation would never have been detected using pooled samples containing many seeds instead of assaying individual seeds, as the mean activity is the same in both types of assays. In situ localization techniques are revealing the enormous range of cell-to-cell variation in gene expression and metabolic activities within tissues that have been considered to be relatively homogeneous. New methods of analysis, including the population-based approaches described here, are required to interpret and understand these data, and more importantly, to design experiments not confounded by averaging. If we assume that hormone sensitivity is determined by specific receptors, then variation in sensitivity might be governed simply by differences in the numbers of such receptors per cell (Rodbard, 1973). The individual receptor molecules might bind the hormone only over a relatively narrow concentration range (Fig. 2C). If so, it would be fruitless to search for a receptor molecule with binding characteristics exhibiting the broad concentration dependence evident in a cell population (tissue) response. Instead, experiments might be directed toward testing whether variation in hormonal response at a cellular level correlates with the presence and amount of putative receptor molecules. For the example of aleurone protoplasts, it may be possible to separate populations of responsive and nonresponsive protoplasts (e.g. as by flow cytometry) and then test the two populations separately for their receptor content. Receptor distributions among cells in tissues might be determined using in situ localization and quantitation techniques (Jones and Herman, 1993). The possibility of sensitivity variation in responsive cell populations will need to be assessed in interpreting growth or developmental responses at the tissue and organ levels.

CONCLUSIONS

Simple observations support the view that variation among cells and tissues in their sensitivities and response times is an integral component of hormonal regulation of plant development. A quantitative model based on sensitivity threshold distributions and proportional rate responses to regulatory factor levels can account for a wide range of phenomena in plant growth and development. An intriguing implication of this model is that biological time may expand or contract depending on the amount by which the concentration of a regulatory factor differs from its threshold level. Developmental or growth rates at a range of regulator concentrations can be normalized on an appropriate biotime scale. Stochastic variation in response thresholds among individual cells may underlie developmental patterns, resulting in a rich plasticity of physiological responses that is not to be confused with experimental error. New experimental designs and analyses based on population statistics will be required to test this model's implications. A matrix of elastic developmental time scales, coupled with stochastic variation among individual cells, tissues, organs, and plants, provides a vantage point for exploring and interpreting many aspects of plant biology.

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